APPENDIX B

TO THE

RESPONSE TO OFFICE ACTION

FILED NOVEMBER 11, 2011

IN THE MATTER OF:
European Patent Application No. 04709824.9
("the European Patent Application")

- AND -

IN THE MATTER OF:
Summonses to Oral Proceedings
4th November 2010 and 3rd January 2011
("the Summonses")

SECOND DECLARATION OF THOMAS DIERKS

I, Thomas Dierks, of Universitaet Bielefeld, Fakultaet für Chemie, Universitaetstr. 25, 33615 Bielefeld, Germany, do hereby solemnly and sincerely declare as follows:

1. I am an inventor in respect of the above-mentioned European Patent Application and am fully aware of its contents. I have prepared and filed an earlier declaration in respect of this case. That declaration is referred to here as "Dierks 1" to distinguish from the present declaration, which can be referred to as "Dierks 2".

2. As discussed in Dierks 1, I have been working in the field of sulfatase deficiencies since 1995 and consider myself competent to comment on what was known in this field at the 11th February 2003 priority date of the International Patent Application on which the present European Patent Application was based. A copy of my CV is attached as Exhibit A, which provides details of my qualifications and experience.

3. I have read the Summonses and the associated comments from Examiner Schneider in respect of this case. I have also read the response now on file and the accompanying amended claim sets (referred to as MR and AR1 to AR9 in the response).

4. I would like to thank Examiner Schneider for his helpful analysis and for explaining why he thinks D2 is the closest document to the invention. I have therefore focused on this document.

5. D2 is a 1998 paper by Andreas Schirmer and Roberto Kolter entitled "*Computational analysis of bacterial sulfatases and their modifying enzymes*". It is essentially a theoretical paper, concerned primarily with bioinformatics, i.e. with the "computational" analysis of sequences. I was well aware of this paper in 2003, as were others in my group and I think it is very important to put it in its true context.

6. The authors of D2 actually did a good job at the time in presenting, on theoretical grounds, candidate genes for **bacterial** formylglycine (FGly) generating enzymes. They did not however present a single eukaryotic or even human gene as a homolog to the bacterial candidates. They were primarily concerned with analysing bacterial genes and associated proteins, as is absolutely clear from the title. What came out from this study was a prediction of FGly-generating enzymes

only for the so-called serine-type sulfatases, which exist only in bacteria but not in eukaryotes.

7. If a non-inventive person without knowledge of the present invention were to have used this paper as a starting point at the time, it is clear that such a person would have tried to use bioinformatics to try to look for a human homolog. Indeed it should again be recalled that the title of the paper refers to "computational analysis" (used in bioinformatics). This was very fashionable at the time and was considered to be a powerful technique. Thus this would clearly have been the obvious way to go at the time if a non-inventive person were to have started from D2 without any knowledge of the present invention.

8. It is now known that D2 presented intelligent but purely theoretical ideas that would have actually failed completely and would have been massively misleading to someone seeking to discover FGE.

9. Indeed it was discovered later on that the candidates discussed in D2 belong to the so-called "radical SAM protein family", which is a bacterial protein family [see e.g. P.A. Frey et al. (2008) The Radical SAM Superfamily. Crit. Rev. Biochem. Mol. Biol. 43:63–88; S.J. Booker and T.L. Grove (2010) Mechanistic and functional versatility of radical SAM enzymes. F1000 Biology Reports 2:52].

10. I feel particularly able to discuss this, because it was my group who first showed that many bacterial sulfatases are modified by radical SAM enzymes or by another (even now) unknown modifying system. The latter, like the radical SAM enzymes, are present in bacteria, but definitely are also unrelated to eukaryotic FGE. I refer here by way of example to two key papers, namely Q. Fang, J. Peng, T. Dierks (2004) "Posttranslational formylglycine modification by the radical SAM protein AtsB." J. Biol. Chem. 279: 14570-14578 and A. Benjdia, G. Deho, S. Rabot, O. Berteau (2007) "First evidences for a third sulfatase maturation system in prokaryotes from E. coli aslB and ydeM deletion mutants" FEBS Letters 581: 1009–1014.

11. I would also like to add that, although the members of the bacterial radical SAM enzyme family work on bacterial sulfatases, they do not work on mammalian sulfatases. These enzymes are sensitive to oxygen and thus stable only under anaerobic or micro-anaerobic conditions, which only exist in bacterial habitats. This is why they have been termed anaerobic sulfatase-maturating enzymes ["anSME", see A. Benjdia et al. (2007b) Anaerobic Sulfatase-Maturating Enzymes: Radical SAM Enzymes Able To Catalyze in Vitro Sulfatase Post-translational Modification. J. Am. Chem. Soc. 129: 3462-3463]. As a consequence, these enzymes are not compatible with the essentially aerobic life of mammalian cells and would be totally useless in treating the sulfatase deficiencies discussed in the present application. By contrast, mammalian FGE is an oxygenase, which uses oxygen for its oxidative function during FGly generation.

12. I would further like to point out that D2 did not elucidate the eukaryotic FGE substrate sequence. Rather, D2 compared from different species a set of sequence regions around cysteine/serine residues to be modified, which showed some conservation. This approach of D2 at the time suggested that only one FGly-generating enzyme existed in nature, which consequently suggested that the bacterial candidate genes discussed in D2 should have orthologs (i.e. homologs with closely related function) in eukaryotes. Both predictions turned out to be completely wrong, as explained above.

13. The position is therefore that, although D2 was regarded at the time as being an interesting paper in respect of bacterial sulfatases, it did not have (and could not have had !) the faintest impact on FGE discovery.

14. I have also carefully reviewed D3, which is a 2001 J. Biol. Chem. paper published by Jens Fey *et al* entitled "Characterisation of posttranslational formylglycine formation by luminal components of endoplasmic reticulum". This has nothing to do with the computational approach discussed in D2. It discusses an assay system. Indeed the focuses of the two papers are completely different. D3 therefore seems to me to be a totally independent document from D2. I can see no reason why a non-inventive person actually starting from D2 would have combined the two at the time, especially if such a person is considered to be cautious and conservative in nature. Furthermore, even if they had been combined this would not result in the present invention anyway. There is nothing in the combination that provides a route to the present invention in any obvious manner.

15. It may also be useful here if I expand a little on Dierks 1. Firstly, I would like to thank the Examiner for agreeing that the methodology that I and my colleagues used, as explained in Dierks 1, was indeed inventive. Secondly, I would like to point out again that the final steps of this methodology did not merely rely on the substrate peptide sequence for development of an affinity matrix. (It should of course anyway be recalled here that the substrate had not been identified in D2, as discussed above.)

16. We actually tried to use the original substrate peptide as an affinity matrix, but this approach failed completely! The FGE activity could not be recovered from this matrix, neither in the bound nor in the unbound fraction. The loss of activity consequently made any further purification impossible and there even was no obvious way at the time of proving whether FGE had bound to the matrix or in fact passed through the matrix with concomitant inactivation. In addition, we found dozens of known proteins, mostly highly expressed ones, to have bound to this peptide matrix, many of them covalently by disulfide bridges, as identified after cleaving these bridges by 2-mercaptoethanol. We thus had to spend a further three years desperately trying to come up with something that would work. Most things we thought of did not work. There was certainly nothing in D2 that would have helped at all (or even in D3 for that matter!)

17. I would add here that I was working with other scientists who were technical experts of worldwide repute, including Mike Heartlein who is named as an inventor on many patent applications and who will be accompanying me to Oral Proceedings in Munich. We had to devise two completely original affinity matrixes and special procedures in order to achieve success.

18. At the outset we had no idea that there would have been such huge obstacles in our way. Furthermore at the time the difficulties arose it was not at all clear how, or even if, they could have been overcome. Indeed in my view a non-inventive person would simply have given up.

19. In summary, I am of the firm view that a non-inventive person simply could not have made the present invention described in the European Patent Application via any obvious route. D2 and D3 are very different documents. Even if they are read together this does not pave the way to the invention or indeed get anywhere close to providing FGE protein.

20. I and my colleagues were well aware of both D2 and D3 and indeed of many other

documents prior to making the invention. If we could have found any obvious route to the invention in any of these documents we would have followed it and this would certainly have saved very many years and considerable stress. However this simply was not possible. In order to be successful we had to devise our own highly original strategy that did not originate either from D2 or D3.

21. I trust that this declaration is of assistance to the Examining Division in putting the invention in context and I would be very happy to provide further assistance, if required, at the Oral Proceedings in Munich.

22. Finally, I confirm that that the foregoing statements are true to the best of my knowledge and belief.

Signed _____ Date: 21st February 2011

Thomas Dierks

[Declared in Bielefeld, Germany]

Exhibit A

I confirm that the attached document is Exhibit A, as referred to in my declaration of today.

Signed _____ Date: 21st February 2011
Thomas Dierks

[Declared in Bielefeld, Germany]